

AMENDMENT

Kindly amend the application, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

IN THE CLAIMS:

Kindly amend the claims, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, to read as follows:

1. (Currently Amended) A method for extracting a soluble or membrane associated intracellular recombinant protein of interest (POI) from a bacterial, yeast or fungal cell, the POI being released from the bacterial, yeast or fungal cell, comprising the steps of:
 - (a) providing a bacterial, yeast or fungal cell comprising a soluble or membrane associated intracellular recombinant POI;
 - (b) releasing the recombinant POI from the cell by contacting the cell with a membrane extracting composition comprising a quaternary ~~quaternary~~ ammonium compound at a concentration of between 0.05% to 0.6% by weight, under conditions sufficient for the release of the recombinant POI in a soluble form; and,
 - (c) recovering the recombinant POI from the membrane extracting composition; wherein the quaternary ammonium compound is selected from the group consisting of Lauroyl Trimethyl Ammonium Bromide (LTAB), Myristyl Trimethyl Ammonium Chloride (MTAC), Cetyl Trimethyl Ammonium Chloride (CTAC), Cetrimide, Cetyl Trimethyl Ammonium Bromide (CTAB), Stearoyl Trimethyl Ammonium Chloride (STAC), Stearoyl Trimethyl Ammonium Bromide (STAB), Benzalkonium Chloride (alkyldimethylbenzylammonium chloride), N-Cetylpyridinium Bromide (N-Hexadecylpyridinium bromide), N-Cetylpyridinium Chloride (N-Hexadecylpyridinium chloride), Benzyl Dimethyl Tetradecyl Ammonium Chloride, Benzyl Dimethyl Hexadecyl Ammonium Chloride and a combination of any two or more thereof, and wherein the recombinant POI is a hexose oxidase (HOX) enzyme.
- 2-3. (Cancelled)
4. (Currently Amended) The method according to claim 1, wherein the membrane

extracting composition comprises from about 0.1% to about 0.5% by weight of the quaternary ~~quaternary~~ ammonium compound.

5. (Currently Amended) The method according to claim 4, wherein the membrane extracting composition comprises from about 0.2% to about 0.45% by weight of the quaternary ~~quaternary~~ ammonium compound.

6. (Currently Amended) The method according to claim 5, wherein the membrane extracting composition comprises about 0.4% by weight of the quaternary ~~quaternary~~ ammonium compound.

7. (Original) The method according to claim 1, wherein the cell is contacted with the membrane extracting composition at temperatures from about 4°C to 40°C.

8. (Original) The method according to claim 7, wherein the cell is contacted with the membrane extracting composition at temperatures from about 20°C to about 30°C.

9. (Original) The method according to claim 8, wherein the cell is contacted with the membrane extracting composition at temperatures from about 25°C.

10. (Previously Presented) The method according to claim 1, wherein the cell is contacted with the membrane extracting composition at a pH of from about 2.0 to about 11.0.

11. (Previously Presented) The method according to claim 10, wherein the cell is contacted with the membrane extracting composition at a pH of from about 5.0 to about 7.0.

12. (Previously Presented) The method according to claim 11, wherein the cell is contacted with the membrane extracting composition at a pH of from about 6.3.

13-22. (Cancelled)

23. (Currently Amended) The method according to claim ~~22~~ 1, wherein the hexose oxidase (HOX) enzyme comprises the amino acid sequence set out in SEQ ID No 23.

24. (Currently Amended) The method according to claim ~~22~~ 1, wherein the hexose oxidase (HOX) enzyme is encoded by ~~[[a]]~~ the nucleotide sequence set out in SEQ ID No 22.

25. (Currently Amended) The method according to claim ~~2222~~ 1, wherein the hexose oxidase (HOX) enzyme is encoded by sequence complementary to a nucleotide sequence capable of ~~hybridising~~ hybridizing to the nucleotide sequence set out in SEQ ID No 22 ~~or a sequence complementary to the hybridisable sequence.~~

26. (Currently Amended) A method for screening for mutated cells or transformed cells producing elevated levels of a soluble or membrane associated intracellular recombinant

POI comprising the steps of:

- (a) growing the mutated cells at 30°C;
- (b) incubating the mutated cells or transformed cells with the membrane extracting composition comprising a quaternary ammonium compound at a concentration of between 0.05% to 0.6% by weight,
- (c) recovering the cell free medium; and
- (d) screening the cell free medium for elevated levels of the intracellular recombinant POI;

such that the presence of the intracellular recombinant POI in the cell free medium is indicative that the intracellular recombinant POI has been released; wherein the quaternary ammonium compound is selected from the group consisting of Lauroyl Trimethyl Ammonium Bromide (LTAB), Myristyl Trimethyl Ammonium Chloride (MTAC), CetylTrimethyl Ammonium Chloride (CTAC), Cetrimide, Cetyl Trimethyl Ammonium Bromide (CTAB), Stearoyl Trimethyl Ammonium Chloride (STAC), Stearoyl Trimethyl Ammonium Bromide (STAB), Benzalkonium Chloride (alkyldimethylbenzylammonium chloride), N-Cetylpyridinium Bromide (N-Hexadecylpyridinium bromide), N-Cetylpyridinium Chloride (N-Hexadecylpyridinium chloride), Benzyl Dimethyl Tetradecyl Ammonium Chloride, Benzyl Dimethyl Hexadecyl Ammonium Chloride and a combination of any two or more thereof, and wherein the recombinant POI is a hexose oxidase (HOX) enzyme.

27. (Cancelled)

28. (Currently Amended) A method of using a membrane extracting composition comprising a ~~quaternary~~ quaternary ammonium compound to extract a soluble or membrane associated intracellular recombinant POI from a bacterial, yeast or fungal cell, the POI being released from the bacterial, yeast or fungal cell, in which the membrane extracting composition comprises a ~~quaternary~~ quaternary ammonium compound at a concentration of between 0.05% to 0.6% by weight and is contacted with the bacterial, yeast or fungal cell and in which the released recombinant POI is recovered from the membrane extracting composition; wherein the quaternary ammonium compound is selected from the group consisting of Lauroyl Trimethyl Ammonium Bromide (LTAB), Myristyl Trimethyl Ammonium Chloride (MTAC), CetylTrimethyl Ammonium Chloride (CTAC), Cetrimide, Cetyl Trimethyl Ammonium Bromide (CTAB), Stearoyl Trimethyl Ammonium Chloride (STAC), Stearoyl Trimethyl Ammonium

Bromide (STAB), Benzalkonium Chloride (alkyldimethylbenzylammonium chloride), N-Cetylpyridinium Bromide (N-Hexadecylpyridinium bromide), N-Cetylpyridinium Chloride (N-Hexadecylpyridinium chloride), Benzyl Dimethyl Tetradecyl Ammonium Chloride, Benzyl Dimethyl Hexadecyl Ammonium Chloride and a combination of any two or more thereof, and wherein the recombinant POI is a hexose oxidase (HOX) enzyme.

29. (Currently Amended) A hexose oxidase (HOX) enzyme producible by a method according to claim 1, wherein the hexose oxidase (HOX) enzyme is encoded by a nucleotide sequence set out in SEQ ID No 22, or a sequence complementary to the ~~hybridisable~~ hybridizable sequence, and wherein the nucleotide sequence is synthesised by the oligonucleotides as set out in SEQ ID Nos 2-22.

30-32. (Cancelled)